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Nutrition and Hookworm Infection: A Causal Analysis of the Literature

A Thesis
Presented to Department of Epidemiology of Microbial Diseases
Yale School of Public Health
of
Yale University
in Candidacy for the Degree of
Master of Public Health
in Epidemiology of Microbial Diseases
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by
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Committee Member: Michael Cappello, MD

May, 2022

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Abstract

Nutrition and Hookworm Infection: A Causal Analysis of the Literature

Mengting Su

2022

Hookworm infection is one of the neglected tropical diseases, particularly in low and middle-income countries, but we know relatively little about causal evidence related to malnutrition. This is mainly due to the relatively small research community compared to those investigating the impact of hookworm infection on host nutritional status and disease complications. Causal inference is critical in epidemiology and biomedical research because it can be a powerful tool in enlightening prevention efforts and developing etiology models. The application of causal criteria to address complex epidemiological research questions is indeed helpful in proposing more interventions to improve the health and well-being of marginalized human populations. Herein, the causal criteria are analyzed across studies differing in their theoretical methods and assumptions under the nutrition-hookworm infection paradigm. We found evidence that supports nutritional deficiency in protein, energy, and/or zinc playing a role in influencing the success of hookworm infections and the ability of the host to respond to infections. Thus, when ascertaining causal relationships, causal inference tools are applicable for future studies related to nutrition and infectious diseases.

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This thesis work is dedicated to my dear parents, Xinguo Su and Kehua Chu, who has been my constant source of unconditional love, backup, support, and encouragement during all the ups and downs in my life. This work is also dedicated to Jiazhe Xu. He has been the source of my strength throughout the last year of graduate school. I sincerely appreciate their belief in me.

Last but not least, this thesis is dedicated to my “Lao Lao” (grandma), who raised me and taught me to be a genuine, strong, and independent woman. I hope she can feel my gratitude in heaven.

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Chapter 1

Introduction

1.1 Background

Hookworm disease or hookworm infection, characterized as soil-transmitted hookworms like *Ancylostoma duodeanale*, *Ancylostoma ceylanicum* and *Necator americanus* infecting human small intestinal lumen, is striking over 500 million people worldwide [1]. Relevant scientific studies and clinical diagnoses have shown that hookworm infections typically cause consequential health problems, including blood loss and anemia, especially iron-deficiency anemia [1]. Previous studies have mainly demonstrated why hookworm infections cause iron-deficiency anemia and malnutrition, but rarely touched on systematically synthesizing causal evidence of the relationship between nutritional status and hookworm infection. In particular, given that nutritional status is hard to measure, and all involved components are complex and diverse, it is difficult to set a stage for causal evidence and associations in epidemiological and biological studies to study the relationship between nutrition and hookworm disease.

Hookworm infection reveals itself as one of the detrimental neglected tropical diseases (NTDs) in the human populations in several ways:

- (a) 576 to 740 million people live in areas endemic for hookworm and are thus at risk of infection [1];
- (b) it affects people living in areas with poor sanitation and hygiene infrastructure and

practices;

- (c) endemic areas are primarily located in tropical regions with warm and moist climates;
- (d) infected people are chronically suffering from morbidity rather than mortality;
- (e) living condition improvements would greatly reduce the burden of hookworm infections, thus it is closely associated with poverty and “the bottom billion” populations [2].

It is known that human hookworm infections are caused by three main species, *A. duodeanale*, *A. ceylanicum* and *N. americanus*. Some species affecting animals, such as *Ancylostoma caninum*, could potentially infect human beings but never reach full reproductive maturity [1]. For hookworm infection, it is well-known that infection can lead to host malnutrition and detrimental health conditions. In resource-poor and underdeveloped endemic countries and areas, undernutrition is a common problem contributing to increased mortality and morbidity. Undernutrition also results in delayed recovery and increased severity of infectious diseases.

1.2 The Life Cycle of Hookworms

Figure 1.1 [1] shows the life cycle of the intestinal hookworm. Hookworm eggs are initially shed in feces by infected hosts to the outside environment. Under ideal environmental conditions, hookworm larvae hatch and grow into free-living forms in the soil. In 6 to 12 days, eggs molt twice and develop into the infectious stage (third stage larvae, or L3). L3 can stay in the soil or on vegetation for up to a month waiting for penetrating the hosts through a percutaneous route (*N. americanus* and *A. duodenale*) or oral ingestion (*A. duodenale*) [3]. Skin penetration can happen without notice, resulting in a local rash called ‘ground itch’. In endemic regions, people who work in the field are susceptible to getting infected with hookworm. Once L3 passes through the skin barrier, they are transported through the circulatory system to the heart and then the lungs. L3 crosses the alveoli and bronchial tree and ascends the trachea. Finally, L3 reaches the pharynx and is introduced to the host gastrointestinal tract. Once larvae find the small intestine, they use their cutting plates

(*Necator* spp.) or teeth (*Ancylostoma* spp.) to feed on blood and host nutrients. It takes about 4 to 6 weeks for hookworm larvae to mature (L5) and reproduce in the small intestine. Finally, eggs produced by L5 would shed with the host feces.

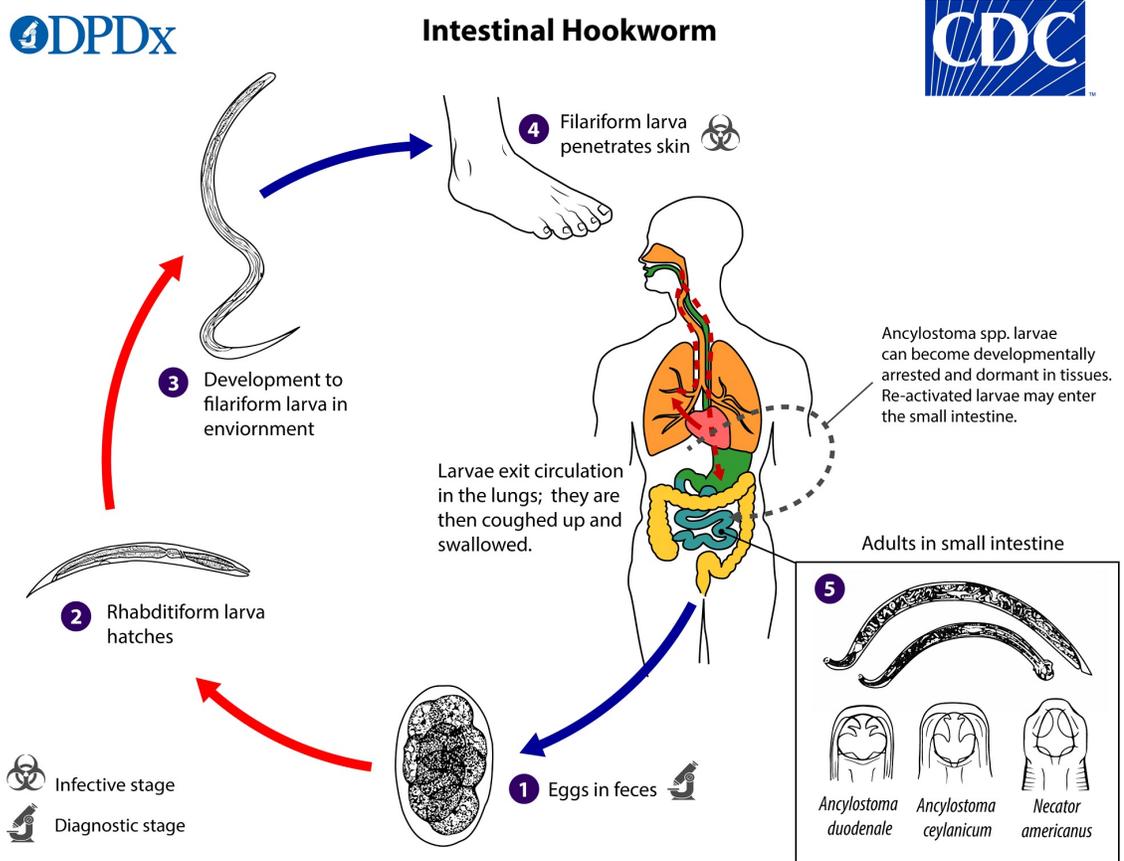


Figure 1.1: Lifecycle of the Intestinal Hookworm (CDC, 2022).

1.3 Human Immunological Response to Hookworm Infection

The complexity of the hookworm life cycle provides pathogens many opportunities to invade human bodies. As a result, both humoral and cellular responses are triggered to defend against hookworm invasion. Immune responses to fight against hookworm infection can be induced in several body locations, including the skin, the circulatory system, the lungs, and the digestive system [4]. Basophils try to trap larvae in the skin and prevent further penetration into the human body. If the hookworm infection progresses and makes it to the circulatory system, systemic or localized eosinophilia would target the hookworm. Mucosal

mastocytosis would help prevent tissue damage. In the lungs, M2 macrophages aim at fighting off the parasites to prevent migration to the small intestine. The clearance of hookworm infection in human bodies is accomplished by reducing the adherence of adult worms to the human intestinal tract and/or complete removal of adult worms from the small intestine.

Most studies demonstrated that the human immune response is primarily induced by the T-helper type 2 (Th2) immune response to defend human bodies from hookworm invasion, following a minor T-helper type 1 (Th1) response [3] [4] [5]. Some studies reported a mixed reaction of both Th1 and Th2 immune responses [6] [7] [8]. Only a tiny proportion of studies showed only strong Th2 immune responses [9]. Although Th2 cell response plays a vital role in fighting against hookworm infection, it is relatively unsuccessful in hookworm clearance [4]. Furthermore, Th2 cytokines, typically IL-4, would mediate antibody response led by isotypes IgG1, IgG4, and IgE to clear hookworm infection in human bodies [4]. In addition, isotypes IgM, IgD, and IgA were shown to be protective in hookworm-endemic subjects for fighting against both the human hookworm *N.americanus* and the zoonotic dog hookworm *A.caninum* [7]. IgM, IgD, and IgA all help with increased clearance of hookworm infections, with IgA being specifically anti-larval and IgD being specifically anti-adult [7].

1.4 Importance of the Study

A frequent theme running through the majority of previous related studies was mainly around the impact of hookworm infection on host nutritional status. The effects of host nutritional status on the complex host-pathogen dynamics during hookworm infection remains important to study. From a global health research perspective, both malnutrition and hookworm infection remain problematic issues in low and middle-income countries (LMICs). Malnutrition in all forms still impacts 2.3 billion people worldwide [10]. Approximately 45% of child deaths are associated with undernutrition, and most of these cases occur for those in developing countries [11]. Protein-energy undernutrition remains a running theme in malnutrition endemic areas. Worldwide, around 20% of the population is at risk of zinc deficiency, with LMICs leading in prevalence [12]. In LMICs, zinc deficiency is one of the

major causes of morbidity and mortality [13] [14]. At the same time, hookworm infection is a widespread disease causing more than 500 million cases worldwide, particularly in developing regions and countries [1].

In addition, both health conditions are social justice problems that reflect health inequity. Although every country is impacted by at least some form of malnutrition, people living with low socioeconomic status (SES) and poverty are more likely to experience it. Without money and resources, people with low SES would have trouble accessing nutritious food. Besides, malnutrition would cause an infinite feedback loop leading to poverty, as all forms of malnutrition increase healthcare costs and hamper productivity and economic gain. Hookworm infection similarly impacts human health and well-being. Farmers who live in developing countries that rely heavily on agriculture would have a higher chance of acquiring hookworm infection, especially by walking barefoot in contaminated soil [1]. Improvements in industrialized agriculture and living conditions would reduce hookworm infection cases substantially. Co-infection of hookworm and other types of diseases remains problematic in endemic regions, leading to a greater health gap worldwide. Thus, it is necessary to call people's attention to address the issues.

Given the incremental nature of public health research, the accumulation of small pieces of evidence from individual studies over the years can lead to significant breakthroughs over time. In science, equivocal research results would lead to poor decision-making in action. Thus, synthesizing and assessing similar as well as contrary results would help recognize good evidence from the existing scientific literature. At the same time, adopting conceptual frameworks and criteria assessment tools allows us to generate information from the entire body of evidence instead of the little evidence from every single study. This review will provide an example of an applied approach for rigorously interrogating existing literature to assess causality in nutrition and infectious diseases.

1.5 Challenges

Given the complex nature of nutrition-and-infection association, challenges remain in studying nutrition and infection in human populations. Specifically, challenges regarding defining

different types of causes, specifying the meaning of malnutrition and infection, as well as choosing the right study types to assess the causal relationship between nutrition and hookworm infections are discussed in this section.

Defining causes and assessing causal relationships remain essential and challenging in the field of epidemiology. In general, an effect is induced by a series of events initiated by a cause itself or in a combination of other causes [15]. Rothman further defined a necessary cause as a condition that must present to lead to an event and a sufficient cause as a condition or a sequence of conditions that is enough to guarantee an event to occur [15]. In the case of hookworm infection, for example, the presence of parasitic hookworm would be a necessary cause of hookworm infection. To fulfill a sufficient cause, it may be contemplated as the onset of early stages of the disease in disease etiology [16]. For instance, for a sufficient cause, smoking would be a component of a sufficient cause for developing lung cancer, but it cannot be sufficient alone. The nutritional status is neither necessary nor sufficient to cause hookworm infection. Malnutrition may be correlated with hookworm infections sometimes, but hookworm infections can happen without the condition of malnutrition. Thus, this study aims at bridging the gap in the relationship between nutrition and hookworm infections. In this study, the term “cause” and “causality” are used to explore the role of nutrition in causing hookworm infections to be more severe and/or making it harder for hosts to expel pathogens.

Moreover, the heterogeneity of both nutritional status and infections implicates many possible meanings. Malnutrition, in definition, indicates all forms of nutritional or energetic deficiencies, excesses, and imbalances [1]. Although the cause of malnutrition varies from case to case, multiple components including socioeconomic status, lack of access to food, lack of education and information, political and social insecurity, war, gender inequity, and/or natural resources could influence one’s nutritional status. In LMICs, the underlying issues could expand to related factors, including lack of access to healthcare, poor sanitation conditions, family food insecurity, and poverty. In certain areas with prevalent malnutrition issues, it is of vital importance to figure out the causes of such conditions to develop health interventions.

Infections represent diseases caused by the presence of pathogens. A patient can get

an infection without symptoms or have a symptomatic disease that is not caused by the pathogen. Over time, infections vary depending on the disease progression, intensity of infection, and whether it is first-time or repeated exposure [17]. These factors would potentially influence host immune response, leading to a complex association between nutritional status and infections. Moreover, most historical public health and clinical research emphasize the role of infections in causing malnutrition rather than the opposite. This is particularly true for malnutrition and hookworm infection interaction studies. Additionally, ethical problems around using human subjects to study the association make it essential to extrapolate evidence from controlled lab studies to human populations. But the difficulty in gathering evidence remains in this process, as analogous animals are not the same as human beings in many different biological ways.

There are also challenges to researching causal pathways in nutrition and infection. Randomized controlled trials (RCTs) are seen as the “gold standard” for causal evidence and are used to evaluate the large-scale impact of health and nutritional interventions [18] [19]. However, under the complex interaction between nutrition and infection, it is hard to depend on RCTs alone to control all the effect modifications in the real world [20]. Concerns around limited external validity arise when applying the evidence from RCTs to nutritional interventions with large populations and complex causal pathways [20]. Two types of effect modifications, both biological and behavioral, must be carefully examined when assessing the generalizability of a randomized controlled trial [20]. In different populations, the dose-response relationships would be different [20]. Meanwhile, when delivering interventions to large populations, the dose would be diversified due to varying behaviors of recipients, contributors, and related facilities [20]. For example, it is difficult to ensure a high compliance rate when delivering health interventions to a large population without special efforts. RCT results would be greatly affected if they were not conducted under “ideal conditions” [21]. Thus, it is important to find another approach to understand and demonstrate the causality other than relying on the probability statement generated from the RCT results. Plausibility and adequacy designs in observational studies would greatly compensate for the problem in evaluating large-scale interventions [21] [22].

Defining probability. Probability assesses causality by analyzing the statistical infer-

ence drawn from RCTs [22].

Defining plausibility. Plausibility evaluation, by ruling out external confounding factors, aims at using observational studies with a comparison group to draw causal relationships [22].

Defining adequacy. Adequacy assesses the program outcome based on the improvement of both/one of the impact and progress indicators made in a time trend fashion [22]. Despite different types of interventions, adequacy assessment looks at if the health indicators have made substantial progress as a whole [22].

1.6 Framework

Building upon the classic host-pathogen interactions model [23], the nutrition-infection conceptual frameworks developed by Humphries, Scott, and Vermund [17] were modified and applied to present the findings of the study. The model describes six potential points of influence for the pathogen and host interaction under the influence of nutritional status [17]. Three of the stages emphasized the influence of nutrition on the pathogen, with the rest of the three deliberating on the impact of nutrition on the host (refer to Table 1.1).

Given that determining and defining the association between two events is central to public health science itself, it is fundamental to develop causal criteria for infectious disease epidemiology. Throughout the history of epidemiology, scientists have utilized conceptual causal criteria to assess the relationship between health risk factors and associated health conditions (refer to Table 1.2). Following Koch's Postulates [24], Sir Austin Bradford Hill [25] further proposed seven causal criteria to study the lung cancer epidemic in the 1960s. Hill's well-known causal criteria soon inspired many scientists in medicine as well as public health to study the association between risk factors and diseases. In the field of nutritional science, Potischman and Weed added biological plausibility to part of the criteria from Hill to assess nutrition-related health topics [26].

In 2016, in order to study the causal relationship between *Plasmodium vivax*, a malaria species, and subsequent nutrition deficiency, Monteiro and fellow researchers combined all previous causal criteria and added analogies from similar studies [27]. In this study, the

Does malnutrition alter the success of the pathogen?	Does malnutrition alter the success of the host?
Exposure – pathogen finds host <ul style="list-style-type: none"> • Spatial & temporal overlap of host and infective stage • Chemical cues that attract pathogen or vector to host • Production and handling practices that lead to pathogens on food 	Immunity & resolution <ul style="list-style-type: none"> • Reduced immune response • Inappropriate type of immune response • Inappropriate timing of immune response
Pathogen establishes in host <ul style="list-style-type: none"> • Crosses barriers (gut, skin, lungs) • Finds preferred site • Develops to reproductive stage • Parasite survives 	Disease severity <ul style="list-style-type: none"> • Increased tissue damage • More severe symptoms • Prolonged symptoms • More systemic consequences
Pathogen proliferates & spreads <ul style="list-style-type: none"> • Pathogen attains nutrients needed to replicate • Viral particles, spores, oocytes or eggs released into environment 	Response to treatment <ul style="list-style-type: none"> • Altered absorption • Altered activation of drugs • Altered half-life of active metabolites

Table 1.1: Nutrition-Infection Conceptual Frameworks (Humphries et al., 2021).

modified version of Causal Inference Criteria Assessment Tools, adapted from Monteiro et al. [27], was used to integrate the causal evidence from previously existing research. The nine classic “aspects of association” for evaluating epidemiological studies were further divided into two broader categories (refer to Table 1.3). Strength, consistency, biological gradient, coherence, and temporality were mainly used to assess epidemiological evidence. While experimental design, plausibility (biological), analogy, and specificity were used to evaluate evidence gathered from clinical, animal, and lab studies.

1.7 Purpose and Objectives

Host nutritional status is essential for both the success of a pathogen and the host’s resolution of the infectious disease [17]. To better understand the complex relationship between nutrition and hookworm infection, it is critical to leverage classical epidemiological principles, causality theories, and different types of causal relationship models to assess disease

Koch's Postulates (1890) Infection focus	Bradford Hill (1964) Cancer/Tobacco focus	Potischman & Weed (1999) Nutrition focus	Monteiro et al. (2016) Nutrition and Malaria
<ul style="list-style-type: none"> • Pathogens present in every case of disease • Pathogen isolated from host with disease • Symptoms /disease reproduced when pure culture of pathogen inoculated into healthy susceptible host • Pathogen recoverable from experimentally infected host 	<ul style="list-style-type: none"> • Strength • Consistency • Specificity • Temporality • Biological gradient • Coherence • Experimental design 	<ul style="list-style-type: none"> • Strength • Consistency • Temporality • Dose Response (biological gradient) • Biological plausibility 	<ul style="list-style-type: none"> • Strength • Consistency • Specificity • Temporality • Biological gradient • Coherence • Experiment • Plausibility (biological) • Analogy

Table 1.2: Summary for Criteria for Causal Inference (Humphries et al., 2021).

progression and plausible routes of malnutrition impacts on an infection. The purpose of this paper is to elucidate the effects of host nutritional status on continuous host-pathogen “negotiation” during hookworm infection. The primary objective of this paper is to gain a better understanding of the causal relationships between nutrition and hookworm infection across studies.

Epidemiological Evidence	Strength
	Consistency
	Biological gradient
	Coherence
	Temporality
Animal Models, Clinical trials, intervention studies	Experimental Design
	Plausibility
	Analogy
	Specificity

Table 1.3: Causal Inference Criteria Assessment (adapted from Monteiro et al., 2016).

Chapter 2

Research Design

2.1 Methodology

To conduct a replicable and precise causal review on the relationship between nutrition and hookworm infection, the protocol for a systematic review developed by Sicotte et al. [28] was utilized for this paper. In addition, features of the review process for conducting systematic reviews in nutrition and public health demonstrated by Lichtenstein et al. [29] and Khan et al. [30] were used. The comprehensive methodology process of literature review involves the following steps:

1. Frame the specific questions to review, identify relevant publications, and assess the need to conduct a causal literature analysis.
2. Write the review proposal and protocol of the literature.
3. Identify review sources through database search, assess the risk of bias and study quality, summarize, and synthesize evidence from studies.
4. Interpret the reviewer's findings and insights.

2.2 Locating Studies/Articles for Review

To identify studies relevant to addressing the research questions, published studies including epidemiological studies, human studies, animal studies, and lab studies have been searched.

In composing this literature review, biomedical research engines Scopus and Google Scholar were utilized as the primary journal databases to gather peer-reviewed research articles with matching keywords relevant to helminth infections and malnutrition (refer to Figure 2.1 and the PRISMA flow chart of Appendices). Epidemiological studies, clinical case studies, clinical case studies, relevant animal and lab studies were all included for final analysis. Scopus through Yale Medical Library web-source was searched for titles containing the free-text terms for hookworm, nutrition, and other interchangeable terms (refer to Table 2.1). All articles on Scopus relevant to search criteria were selected for further evaluation. Additional search on google scholar was used to search for articles related to hypotheses. This search strategy is also supplemented by reviewing references listed in the identified relevant articles. The full text of all relevant and potentially relevant publications was retrieved simultaneously.

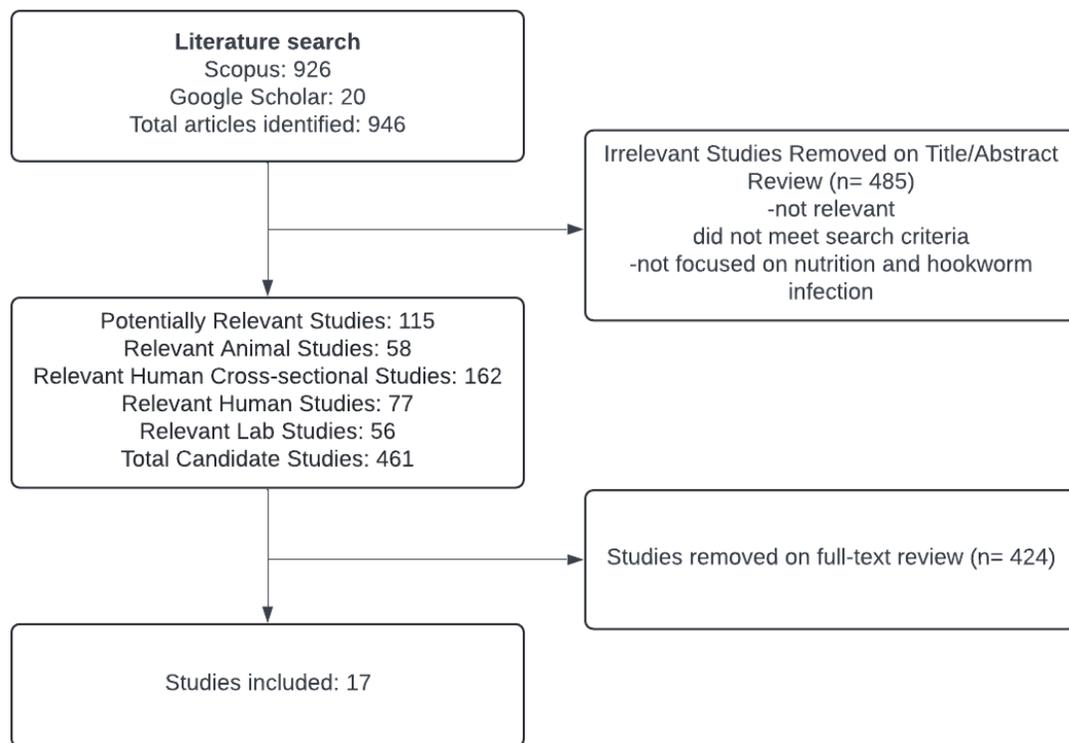


Figure 2.1: Flowchart of the Systematic Literature Review.

Hookworm-related searching terms	‘Hookworm’, ‘Soil-transmitted helminth’, ‘STH’, ‘Helminth’, <i>‘H. polygyrus’</i> , <i>‘N. brasiliensis’</i> , <i>‘A. ceylanicum’</i> , <i>‘H. bakeri’</i>
Nutrition-related searching terms	‘(Host) nutrition’, ‘Malnutrition’, ‘HAZ’, ‘WAZ’, ‘BAZ’, ‘WHZ’, ‘Macronutrient’, ‘Micronutrient’, ‘energy deficiency’, ‘vitamin A’, ‘protein’, ‘energy’, ‘iron’, ‘selenium’, ‘vitamin E’, ‘zinc (deficiency)’, ‘(Micro)-nutrient supplements’
Interchangeable words for the key terms	‘(Host) nutrition / malnutrition / (macro- or micro-) nutrient / energy deficiency / HAZ, WAZ, BAZ, WHZ’, ‘Immune response / Th2 / IL-4’, ‘Fecal egg output / worm burden / worm fecundity’, ‘Hookworm / soil-transmitted helminth’ , ‘Micronutrients / macronutrients in place of specific nutrients listed or vice versa’, <i>‘H. polygyrus’</i> , <i>‘N. brasiliensis’</i> , <i>‘A. ceylanicum’</i> , <i>‘H. bakeri’</i>

Table 2.1: Search Terms (acknowledge the sources provided by Molly McLaughlin).

2.3 Study Selection and Evaluation

The eligibility of the studies was evaluated based on a set of predefined inclusion, exclusion, and ranking criteria (refer to Table 2.2). To begin, the abstracts of the literature were evaluated in the first place to determine relevancy. A full-text review was followed, and irrelevant papers that met one of the exclusion criteria were further excluded. Generally, studies were excluded because they were mainly focused on the impact of hookworm or helminth infection on hosts’ nutritional status, on nutrient deficiencies other than the risk factor of interest, or other concepts unrelated to the causal effect of malnutrition.

Endnote and Excel were used to collect and manage the useful data extracted from the studies. In the Endnote library, articles were sorted based on study types and methodologies.

Selection criteria		Scopus & Google Scholar
Inclusion		Peer-reviewed journal articles, review articles, book chapters with relevant matching keywords;
		No publication time frame restriction;
		No geographical or population restrictions;
		Full-text available in English.
Exclusion	Keyword screening process	Articles without matching keywords were excluded.
	Abstract screening process	Studies not relevant to the research questions were excluded.
	Full-text screening process	Studies with comparisons not related to our risk of interest (e.g. protein, energy, zinc) were excluded;
		The outcome of interest not related to hookworm infection was excluded;
Studies that are not relevant to both nutrition and helminth infection were excluded.		
Ranking	Relevant	Sources closely related to the research questions.
	Potentially relevant	Articles related to nutrition and helminth infection but did not address the causal relationship between nutritional status and helminth infection.
	Irrelevant	Studies that were not relevant to protein/energy/zinc deficiency, or did not measure the relationship between nutrition and helminth infection.

Table 2.2: Inclusion, Exclusion, and Ranking Criteria.

Based on the evaluation criteria provided (refer to Table 2.3), papers that fit our goal were read thoroughly. Information including author’s name, year of publication, findings, epidemiological evidence (including strength, consistency, biological gradient, coherence, and temporality), and animal models, clinical trials, intervention studies evidence (including experimental design, plausibility, analogy, and specificity) was extracted from each study and collected in Excel spreadsheet for each hypothesis (refer to Tables of Sources Searched in Appendices). All information in the Excel spreadsheet was recorded based on our notes and conclusions for the key findings. The final conclusive results of this systematic/literature review were based on the sorted eligible studies.

Evaluation criteria categories	High	Moderate	Low
Methodological quality	Little or no bias; results are valid and clear.	Some bias exists, but without validation to the results.	Significant bias and invalid results.
Causality	Address causal inference criteria; results are strongly supported by the causal evidence.	Address at least one causal inference criterion; no indicators to invalidate the results.	Do not address causal inference criteria; invalid results.
Applicability	Sample is applicable to the pre-defined targeted population.	Sample is applicable to subgroups of the pre-defined targeted population.	Sample is a representation of a narrow population of the pre-defined population.

Table 2.3: Evaluation Criteria (adapted from Lichtenstein et al., 2008).

Chapter 3

Results

3.1 Malnutrition Affects the Success of the Pathogen

Malnutrition is a complex condition with many causes, and some of those causes overlap with the conditions that increase the risk of hookworm infection. This has remained the main complication in defining the causality between undernutrition and hookworm infection. Other uncontrollable factors, including co-infection with other infectious diseases, re-infection, individual treatment history, and genetics, would potentially confound the results [4].

The continuing pathogen-host “negotiation” would make it hard to assess the impact of malnutrition on infection [17]. Firstly, pathogens need to find the host in order to invade the host through a breach of primary barriers. For example, many infectious diseases are transmitted through food either from direct exposure or food contamination. Once pathogens find their way to cross biological barriers, such as skin, gut, and mucosa membranes, they find the ideal sites to live, survive, and mature into reproductive stages. Different pathogens target different tissues. Sometimes, nutrition is essential in tissue adherence. For *Trichomonas vaginalis*, iron plays an important role in synthesizing key adhesion molecules to the binding site of the vagina [31] [32]. Besides finding ideal living conditions and establishing in the host, reproduction and proliferation become essential to further spread out new pathogens in the host and to the external environment. Sufficient nutrients are needed to provide energy for pathogen reproduction and the spread of infectious diseases.

3.2 Malnutrition Affects the Success of the Host

Natural recovery from a parasitic infection refers to when the host expels the established pathogen by itself. Nutritional status had been known to be a major player in influencing the host's ability of parasite clearance. Regarding the ways in which malnutrition affects the success of the host, immunity and resolution, disease severity, and response to treatment are three major causal pathways. As nutritional status is closely related to the immune response to infection, malnutrition would potentially lead to reduced or delayed immune response, or even inappropriate types of immune response [4]. In the 1990s, Udani suggested that protein-energy deficiency is related to the delayed immune response to the tuberculin skin test after getting vaccinated against tuberculosis in children [33].

In addition, certain nutrients are essential mediators of diseases, as host tolerance is impacted. For example, a study conducted by Haley and Gaddy showed the association between iron deficiency and *Helicobacter pylori* infection [34]. Iron remains important in up-regulating high iron affinity transporters for *H.pylori* to get enough iron [34]. Subsequently, high iron affinity transporters are correlated with increased pathogenicity of *H.pylori* [34]. Increased tissue damage and more severe symptoms can be influenced by malnutrition. Prolonged symptoms and more severe consequences are possible for progressed disease severity.

Moreover, altered absorption and/or activation of drugs are possibly challenged by malnutrition, as nutrient deficient individuals' ability to respond to drugs is greatly affected [4]. For example, enzymes like cytochrome P450 (CYP) play a vital role in the pathway of drug metabolization, activation, and clearance [35] [36]. In an animal study, there was less cytochrome P450 3A (CYP3A) in low-calorie intake mice compared to the control group [37]. In another study, iron supplementation was shown to be effective in increasing CYP3A4 counts in patients on hemodialysis [38].

3.3 Malnutrition and Hookworm Infection – Findings of Literature Review

For this study, the association between malnutrition and hookworm infection was assessed using different studies. The significance of the three hypotheses (refer to Table 3.1) was assessed using the modified version of Causal Inference Criteria Assessment Tools. Besides epidemiological and clinical evidence, animal model organisms and helminth similar to hookworm were considered analogously to assess the relationship.

Hypothesis 1	Host protein and energy undernutrition, as measured by low anthropometric indicators such as HAZ, WAZ and BAZ scores in human studies, can increase the risk of infection with hookworm.
Hypothesis 2	Host protein and/or energy deficiency (as measured by low HAZ/BMIZ/WAZ) impairs/suppresses the clearance of hookworm infection.
Hypothesis 3	Host zinc deficiency impairs/suppresses the clearance of hookworm infection.

Table 3.1: Hypotheses of the study.

3.4 How Does the Evidence Stand Up to the Causal Criteria?

In terms of nutrition-hookworm infection association, various studies were conducted from the 1960s up to date, adopting different methods, to provide causal evidence to prove the correlation is not due to chance. In this paper, related findings from previously published journal articles were revealed, organized, and discussed using the modified version of Causal Inference Criteria Assessment Tools. As previously identified, nine components of causal guidelines adapted from Monteiro and fellow researchers were used for assessing the causal relationship between nutrition and hookworm infection: 1) strength, 2) consistency, 3) biological gradient, 4) coherence, 5) temporality, 6) experimental design, 7) plausibility, 8) analogy, and 9) specificity [27]. It is worth noting that these criteria originated from inductive methodology, but their popularity needs to be carefully discussed in actual practice [39].

(1) Strength.

Hill originally proposed that stronger associations are more probable to be causal than weaker ones. Although weak associations do not entirely rule out casual relationships, they are more likely to be explained by undetected biases [16]. Only one study that assessed energy malnutrition as low HAZ/BMIZ/WAZ as a risk factor for increased risk of hookworm infection was available. In the Philippines, a cross-sectional study assessing the causal relationship between energy deficiency and hookworm infection among children and adolescents who are aged 6 to 14 years old showed a borderline association between the prevalence of hookworm infection and a low energy intake (21 %, $p=0.05$) [39].

(2) Consistency.

Ideally, associations observed from the studies conducted in different geographical locations among different populations would show consistent evidence. Consistency would also increase the level of confidence that the association between nutrition and hookworm infections is not due to chance. However, it is not possible to strengthen the actual effect solely based on only one epidemiological study.

(3) Biological gradient.

Under certain circumstances, minor exposure would cause the effect. In others, as the exposure level increases, the incidence of the effect increases. This is referred to as a biological gradient, or a unidirectional dose-response effect curve. In all identified articles, no epidemiological study is applicable to assess biological gradients.

(4) Coherence.

Host malnutrition is believed to change the rate of hookworm transmission and the intensity of hookworm infection by hampering host immune functions [40]. According to Papier and fellow researchers, energy deficiency is associated with hampered host immune response against hookworm in children and adolescents and children in the Philippines [39]. Malnutrition-correlated hookworm infections seem, indeed, coherent

with the current knowledge respecting the detrimental effect of nutrient deficiencies, leading to stronger causal evidence between the two events.

(5) Temporality.

In this study, temporality indicates that protein, energy, or zinc deficiencies precede the hookworm infection. Unfortunately, evidence is limited in existing epidemiological studies up to date, as the only available study design was cross-sectional studies. In cross-sectional studies, it is not applicable to assess temporality because cross-sectional studies only collect data at a given point in time.

(6) Experimental design.

Rather than a causal guideline, the experimental design uses clinical, animal, or intervention study methods to assess the relationship between nutrition and hookworm infection. For undernutrition-related hookworm infections, it would be unethical to conduct randomized control trials on healthy human hosts to study malnutrition or hookworm infection.

Only one clinical trial recruiting human volunteers with heavy worm burden was available to study the impact of protein repletion on the clearance of hookworm infection [41]. The difference between the overall hookworm counts before and after dietary treatment was not statistically different in this study [41].

Although evidence gathered from animal studies is not comparable to human studies, as very distinct exposure levels would occur in animal and human hosts; animal studies are still legitimate and valuable sources of evidence assessment under the nutrition-infection paradigm. Until now, no experiment has been performed on non-human primates in analyzing the correlation between nutrition and hookworm infection. More information with regards to animal studies will be discussed in the analogy section.

(7) Plausibility.

Previous immunological studies demonstrated that antibodies are proteins that play an essential role in the systemic Th2 immune response to fight against hookworm

infection [5] [4] [6]. The old findings suggested that protein deficiencies in the diet might impede the production of necessary antibodies to combat hookworm infection, making it harder to clear the infection. A study done by Pacanaro et al. showed a decreased production of IgG in low protein diet-fed hamsters, making it hard to expel worms from the host [42]. Additionally, compromised immune response, with significantly lower serum parasite-specific IgG1 and lower eosinophil, was shown in low protein-fed mice [43].

Boulay and fellow researchers also suggested that hampered immune response, with significantly lower eosinophilia, was shown in low zinc-fed mice [43]. Having an impaired immune system would weaken parasite clearance in the host. In a study conducted by Shi et al., the findings demonstrated that energy deficiency hampered IL-5 production in the early phase of the infection and reduced the DTH response in the late phase of the infection [44]. Again, the compromised immune response was shown in energy deficiency mice with lower IgE and eosinophilia levels, leading to impaired parasite clearance [44]. Shi and colleagues also found that the reduced production of IL-4 and IFN- γ , the reduced peripheral eosinophilia, and reduced serum levels of IgE and IgG1 in mice were attributed to the zinc deficiency [44]. Thus, findings from animal studies indicated that the role of energy, protein, and zinc deficiency in the etiology of hookworm infection is biologically plausible.

(8) Analogy.

Although analogy was often the neglected criteria among all when assessing causation in many previous studies, Weed suggested that analogy can be helpful in associating causal relationships [45]. In this study, analogy plays an important role as a useful assessment criterion. Because the performance of experimental studies on human subjects to study malnutrition and hookworm infection involve issues related to ethics and logistics, animal studies using hamsters and mice would be most appropriate. The diseases most like hookworm infection are analogous to helminth infections caused by other parasites, including *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*, etc. Animal studies using animal hookworm infections and/or animal helminth

infections to prove the causal relationship between nutrition and hookworm infection would increase the likelihood of causation.

In the research which focused on studying protein and/or energy deficiency and its impact on helminth establishment, mice were used analogously to human beings [46] [47] [48] [49]. Infection caused by *N.brasiliensis* [49] and *H.polygyrus* [46] [47] [48] were used analogously to hookworm infection. Results suggested that increased worm burdens were shown in mice fed with protein and/or energy-restricted food [46] [47] [48] [49].

For studies that undertake the relationship between protein and/or energy deficiency and host worm expulsion ability, hamsters [42] and mice [43] [44] [50] [51] [52] [53] [54] were used analogously to human hosts. *N.brasiliensis* [50] [51] [52] [53] [54] and *H.polygyrus* [43] [44] were used as analogously to parasitic hookworm. Findings from animal studies demonstrated that protein and/or energy-deficient mice showed impaired immune response against helminth infection, leading to impaired worm clearance [42] [43] [50] [51] [52] [53] [54].

For zinc deficiency status, two studies used mice and *H.polygyrus* as analogous to humans and hookworm [43] [44]. As a result, compromised immune response and resolution to *H.polygyrus* were shown in low zinc fed mice [43] [44].

(9) Specificity.

A causal relationship is possible if a specific population shows no outcome other than hookworm infection caused by a particular nutrient deficiency of interest at a specific geographic location. More specific associations between exposure and outcome would increase the likelihood of causation. However, this guideline is rather invalid since no strict one-to-one relationship exists between exposure and outcome in human studies. Undernutrition would lead to various health outcomes. Similarly, hookworm infection also has many different infectious and non-infectious causes and risk factors. The complication and diversification of underlying factors would make it hard to establish a one-to-one relationship between undernutrition and hookworm infection.

Whereas in some experimental animal studies, specificity may be clear due to good

control during the design and experimental processes. In many identified animal studies, the model organisms showed compromised immune response against helminth infection when no other explanation exists besides protein, energy, or zinc deficiency diets [42] [43] [44] [46] [47] [48] [49] [50] [51] [52] [53] [54], leading to stronger causal effect.

Chapter 4

Discussion

4.1 Summary of Findings

In this study, a total of seventeen relevant peer-reviewed journal articles (refer to Tables of Sources Searched in Appendices) were identified and selected to unpack how to approach the association between nutritional status and hookworm infection. These are important public health issues, especially in tropical areas and LIMCs, to draw people's attention to address the neglected problems of malnutrition and hookworm infection. Three hypotheses (refer to Table 3.1) regarding malnutrition and hookworm infection were analyzed using nine causal criteria (refer to Table 1.3).

This review demonstrates the complex nature of malnutrition, specifically with a focus on protein, energy, and zinc deficiency, as well as hookworm infection in human populations. The limited number of existing relevant epidemiological studies shows how challenging it is to answer in clarity in any human population, given ethical constraints. The only available human study identified was a cross-sectional study. Animal studies are thus a compelling source of information for addressing the nutrition-hookworm questions. By applying causality theories, animal studies using model organisms and helminth analogous to hookworm are available for further exploration.

4.2 Limitations of Findings/Study

Due to a limited number of relevant studies, especially epidemiological studies and clinical trials, it became difficult to draw a strong conclusion for each hypothesis. For hypothesis 1, there are only two animal studies that are relevant to the question. Though the findings showed a significant association between protein and/or energy malnutrition and increased hookworm risk, it is impossible to conclude that evidence gathered from animal studies is representable to human studies. In two potentially relevant studies, stunting is related to a higher reinfection rate among children in rural Panamá and rural Malaysia [55] [56]. Although stunting, which represents energy deficiency measured in height-for-age z-scores, is a significant predictor of hookworm reinfection, other possible confounding factors still cannot be ruled out.

For hypothesis 2, relevant animal studies were mainly conducted from the 1970s to the 1990s. Interestingly, in 1971, one clinical study done by Tripathy and colleagues showed protein deficiency was not significantly associated with hosts' ability to expel hookworm infections [41]. The result is in contradiction to all other animal studies which showed a significant association between protein malnutrition and hookworm expulsion from the host.

Here, we indicated that diets with low zinc may impair animals' immune response against helminth infection. No epidemiological study was available to address hypothesis 3. In one study conducted in Cambodia, hookworm-infected children showed lower hair zinc concentrations than those who were not infected [57]. This association showed to be borderline significant in the cross-sectional study ($\beta=0.233$, $p=0.051$) [57], suggesting that zinc deficiency may contribute to a higher hookworm burden in the population.

Besides, the quality of evidence analysis may be influenced due to the subjective nature of literature selection and evaluation. Future research should adopt a systematic searching approach in different journal article databases. Despite all these limitations, the current review utilized previous journal articles with the application of causal inference tools to provide a unique perspective on the relationship between nutrition and hookworm infection.

4.3 Challenges and Relevant Recommendations for Further Research

Even though the evidence demonstrates a strong association between malnutrition and hookworm infection was mainly obtained from animal studies, the impact on human populations is still somewhat subjunctive. The number of existing pieces of literature with epidemiological designs in human populations is small, making it hard to draw an indicative conclusion. The only sorted observational article has a borderline significance. Besides, protein deficiency showed no significant effect on hookworm clearance in the clinical study. These all leave the causal relationship between malnutrition and hookworm infection in human populations in doubt. Thus, more causal evidence from epidemiological and clinical research is needed to fill in the literature gap under the nutrition and hookworm infection paradigm.

Chapter 5

Conclusions

5.1 Conclusion

Causal inference is critical in the field of epidemiology and biomedical research because it can be a powerful tool in enlightening prevention efforts and developing etiology models. When addressing complex causal relationships like malnutrition and hookworm infection, causal inference can serve a more useful role than statistical associations. The claim that causal evidence from only RCTs cannot be relied on to provide useful causal association in larger populations was thought-provoking. Clearly, every single method in epidemiology has its potential limitations. Herein, a number of studies differing in their theoretical methods and assumptions were analyzed to avoid drawing conclusions from causal inference studies using a single method. We showed that nutritional deficiency in protein, energy, and/or zinc may contribute to increased hookworm burden, using existing evidence that proposes a role for malnutrition in the etiology of hookworm infection in animal models and human populations. In the meantime, more epidemiology literature is needed to draw stronger causal claims.

Moreover, using animal studies, based on the adapted causal inference tools from Monteiro and fellow researchers, may provide insightful and valuable causal information with respect to hookworm infection causation. The application of causal criteria to address complex epidemiological research questions is indeed helpful in proposing more interventions to improve the health and well-being of marginalized human populations. Besides conscien-

tiously gathering and interpreting good quality evidence, it is also important to take careful caution and self-critical circumspection when making causal claims in research and public health.

All in all, the public health implications of this study remain insightful and significant, as high-quality epidemiological causal claims were proposed to be measured in a more comprehensive way.

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Appendices

Appendix A. Reflection on Learning Process

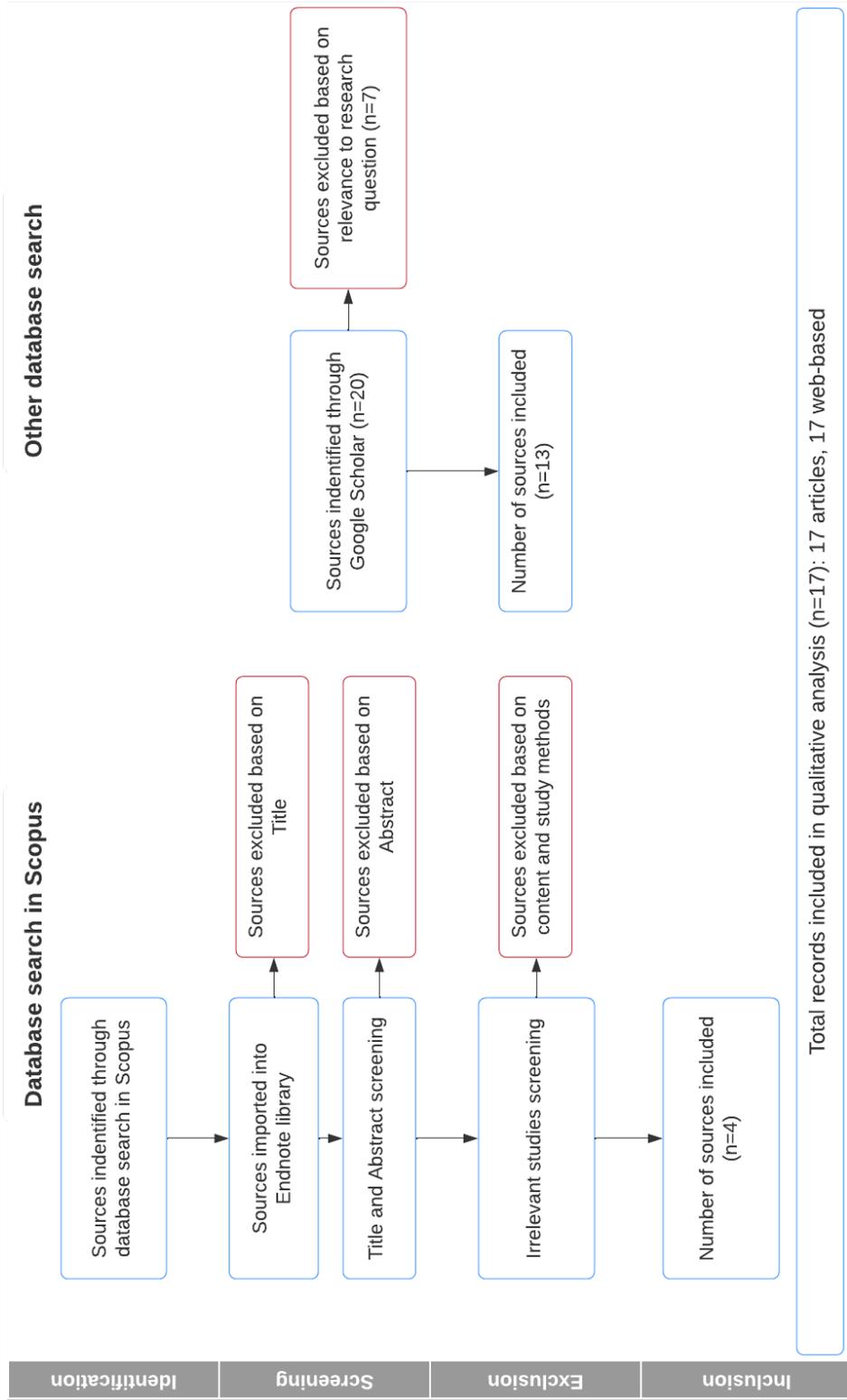
After a year of online school, I picked up this project with limited prior knowledge and understanding of malnutrition and hookworm infection last August. Dr. Humphries provided me with background information to help me understand more about nutrition and hookworm infection. I read the attached articles from her email and took notes carefully. In parallel, I downloaded Endnote to get the shared library. As someone who is terrible at technology, I learned to master another useful academic tool.

The critical turning point of my learning process happened in September. I remember staring at more than 900 articles listed in the Endnote library with no clue where to start, so I seek help from Dr. Humphries. She suggested reading the lab studies that focus more on biochemical pathways and immunological responses. At the same time, I started drawing diagrams to break down the hookworm life cycle and the pathways from the Nutrition-Infection Conceptual Frameworks. I also reached out to my colleague, Zili Zhou, for suggestions on the immunology textbook. Luckily, both Dr. Humphries and Zili provided me with good sources of learning material. After a period of rigorous learning, the whole picture became clear to my mind.

The following challenge, one of the biggest challenges, during the learning process was self-doubt. I kept telling Dr. Humphries that I could not find relevant articles in all identified articles. I could not believe that from over 900 articles gathered from Scopus, I could only find 4 relevant and 3 potentially relevant articles. Dr. Humphries told me that one of the things she learned during her Ph.D. training was not to doubt that you are right when only limited evidence exists. I thought it was because of me that I could not find articles to support causal claims. I was wrong, but I learned from it and laughed at it.

This learning reflection piece is written on the date I turn in my thesis to my advisors. Everything will come to an end. Now that I look back to last year, I would say it is a blessing. I was allowed to go back to school and take a mental break from worrying about my future. I was often too afraid of getting left behind on the way down the path, never really knowing where it led me. When I stopped rushing and started seeking help, I got to see better whether it was the path I wanted to go down. Dr. Humphries was always there for me to support and guide me in the possible directions, rather than giving me specific instructions on how to do research. I learned from her, and I learned how always to use my time to introspect. Knowing where my true passion rests and where it should lead me, I became more steadfast and confident, and no longer stuck with the fear of losing.

Appendix B. PRISMA Flow Chart



Appendix C. Tables of Sources Searched

Summary of Evidence for Hypothesis 1

Host protein and energy undernutrition, as measured by low anthropometric indicators such as HAZ, WAZ and BAZ scores in human studies, can increase the risk of infection with hookworm.								
Title (Author, Year)	Study Design	Findings	Notes	My conclusions	Animal Models, Clinical Trials, Intervention Studies			
					Experimental Design	Plausibility	Analogy	Specificity
Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein-malnourished mice. (Ing et al., 2000)	animal study	Protein-deficiency mice have significantly higher worm burden compared to control and marginal protein groups through both primary (P = 0.024) and challenge (P <0.0001) infection. The results support that protein deficiency increase the survival of worms by hampering intestinal and systemic Th2 responses (reduced IL-4).	Female mice were randomized to three kinds of food with different protein levels, control (24%), marginal (7%), and deficient (3%). Besides protein, rest of the nutrients are identical. Mice are infected with Heligmosomoides polygyrus through primary and challenge infections.	Protein-deficiency mice have significant higher worm burden compared to control and marginal protein groups.	An animal model using mice to study protein-malnutrition and Heligmosomoides polygyrus infection was established in this study.	The intensity and rapidity of Th2 cytokine (IL-4, IL-5, IL-10) and effector responses (eosinophilia, IgE, mastocytosis) combined with limited Th1 responses (IFN- γ , macrophage activity) are essential when it comes to fight against intestinal parasites in both gut and systemic tissues.	Mice are used analogously to human beings. Heligmosomoides polygyrus infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.
Heligmosomoides polygyrus (Nematoda): the influence of dietary protein on the dynamics of repeated infection. (Slater & Keymer, 1986)	animal study	The findings demonstrated that the host nutritional status have an impact on the population dynamic of parasitic infection. Protein-deficient mice have increased risk of infection with H.polygyrus, the effect being evident on both the numbers (higher number of worm count in hosts fed the 2% protein diet than 8% protein diet) and fecundity of adult worms (female fecundity of female worms was higher in hosts fed the 2% protein diet than 8% protein diet, p<0.01).	An analogous study using mice and H.polygyrus.	The findings suggested that the host nutritional status has impact on helminth infection. Protein deficient mice had both higher worm burden and higher fecundity of adult worms.	An animal model using mice to study protein-malnutrition and Heligmosomoides polygyrus infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. Heligmosomoides polygyrus infection was used analogously to hookworm infection.	Specificity was partial clear in this experimental animal study.
The influence of protein deficiency on immunity to Heligmosomoides polygyrus (Nematoda) in mice. (Slater & Keymer, 1988)	animal study	The findings suggested that protein malnutrition hampered the effectiveness of immunization of mice against an intestinal nematode.	An analogous study using mice and H.polygyrus.	Protein malnutrition was found to cause a significant depression in immunity (slightly lower IgG and significantly lower eosinophils), leading to increased risk of infection with H.polygyrus.	An animal model using mice to study protein-malnutrition and Heligmosomoides polygyrus infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. Heligmosomoides polygyrus infection was used analogously to hookworm infection.	Specificity was partial clear in this experimental animal study.
Hypoalbuminemia in energy-malnourished rats infected with Nippostrongylus brasiliensis (Nematoda). (Lunn. P.G., et al., 1988)	animal study	Data shows significant results regarding the association between energy malnutrition and parasite infection (p<0.01).	Restricted-energy food fed and well-fed control rats were infected with a hookwormlike intestinal parasite, Nippostrongylus brasiliensis, to determine the relationship between energy-malnutrition and parasite infection.	In this study, increased Nippostrongylus brasiliensis infection was shown in rats fed with energy-restricted food.	An animal model using rats to study energy-malnutrition and Nippostrongylus brasiliensis (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Rats are used analogously to human beings. Nippostrongylus brasiliensis infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.

Summary of Epidemiological Evidence for Hypothesis 1

Host protein and energy undernutrition, as measured by low anthropometric indicators such as HAZ, WAZ and BAZ scores in human studies, can increase the risk of infection with hookworm.									
Title (Author, Year)	Study Design	Findings	Notes	My conclusions	Epidemiological Evidence				
					Strength	Consistency	Biological Gradient	Coherence	Temporality
Childhood malnutrition and parasitic helminth interactions. (Papier, K., et al., 2014)	cross-sectional study	Children and adolescents who met the nutrient and energy requirement (RENI) had a lower hookworm infection prevalence than those who did not meet the requirement.	Low HAZ/BMIZ/WAZ was used to measure nutritional status. Dietary intake data was collected using a 24-hour recall method from all participants. It was done in the Philippines. A total of 693 subjects include children and adolescents (age 6-14). 667 subjects were infected with hookworm.	In this study, energy deficiency in children and adolescents led to an increased prevalence of hookworm infection.	The percentage of hookworm-infected children and adolescents was lower among subjects who met the energy requirement (21%, p=0.05).	The findings are consistent with other studies.	The study design is not applicable to assess biological gradient.	The findings are coherent with the evidence which support that nutritional deficiency can hamper the body's immune function.	The study design is not applicable to assess temporality.

Summary of Evidence for Hypothesis 2

Host protein and/or energy deficiency (as measured by low HAZ/BMIZ/WAZ) impairs/suppresses the clearance of hookworm infection.								
Title (Author, Year)	Study Design	Findings	Notes	My conclusions	Animal Models, Clinical Trials, Intervention Studies			
					Experimental Design	Plausibility	Analogy	Specificity
Suppression of rejection of <i>Nippostrongylus brasiliensis</i> in iron and protein deficient rats: effect of syngeneic lymphocyte transfer. (Cummins et al., 1978.)	animal study	Depression of the immune response has been observed in protein deficiency rats.	analogous study using mice and <i>N. brasiliensis</i>	An animal study proved that protein-deficient mice have impaired clearance of <i>N. brasiliensis</i> .	An animal model using rats to study protein-malnutrition and <i>Nippostrongylus brasiliensis</i> (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. <i>Nippostrongylus brasiliensis</i> infection was used analogously to hookworm infection.	Specificity was partial clear in this experimental animal study.
Effect of iron and protein deficiency on the expulsion of <i>Nippostrongylus brasiliensis</i> from the small intestine of the rat. (Bolin et al., 1977)	animal study	A significant delay of <i>N. brasiliensis</i> clearance in protein malnutrition, iron malnutrition, as well as protein and iron-deficient animals was shown in the study.	analogous study using mice and <i>N. brasiliensis</i> , primary infection	An animal model proved that protein-deficient mice have prolonged expulsion of <i>N. brasiliensis</i> .	An animal model using rats to study protein-malnutrition and <i>Nippostrongylus brasiliensis</i> (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Rats infected with the nematode <i>Nippostrongylus brasiliensis</i> .	Specificity was partial clear in this experimental animal study.
Dietary protein and zinc restrictions independently modify a <i>Heligmosomoides polygyrus</i> (Nematoda) infection in mice. (Boulay et al., 1998)	animal study	Worm counts were significantly higher in low protein fed mice and low-zinc fed mice. Parasite egg output was significantly higher in mice fed both low protein and low zinc diets. Significant lower serum IgG1 and lower eosinophilia was found in low-protein fed mice.	an analogous study using mice and <i>Heligmosomoides polygyrus</i> (Nematoda)	Compromised immune response was shown in low protein fed mice (significantly lower serum parasite specific IgG1 and lower eosinophil), leading to impaired parasite clearance.	An animal model using mice to study dietary zinc restriction and <i>Heligmosomoides polygyrus</i> (Nematoda) infection was established in this study. This study combined 3 dietary protein levels and 2 dietary zinc levels during both primary infection and a challenging infection protocol.	Compromised immune response was shown in low protein fed mice (significantly lower serum parasite specific IgG1 and lower eosinophil), leading to impaired parasite clearance.	Infective 3rd-stage larvae (L3) of <i>H. polygyrus</i> were obtained by culturing the feces of chronically infected stock CD1 mice on moist filter paper for 7 days.	Under well and carefully control, specificity was clear in this experimental animal study.
Zinc deficiency impairs T cell function in mice with primary infection of <i>Heligmosomoides polygyrus</i> (Nematoda). (Shi et al., 1994)	animal study	The findings demonstrate that energy deficiency (rather than zinc deficiency) hampered I L-5 production in early phase of the infection (by week 2) and reduced the DTH response in late phase of the infection (by week 4).	analogous study using mice and <i>Heligmosomoides polygyrus</i> (Nematoda)	Compromised immune response was shown in energy deficiency mice (lower IgE and eosinophilia level), leading to impaired parasite clearance.	An animal model using mice to study energy deficiency and <i>Heligmosomoides polygyrus</i> (Nematoda) infection was established in this study.	The findings suggest that the normal functions of Th1 and Th2 cells were significantly impaired, making it difficult to expel all the worms in the host. Compromised immune response was shown in energy deficiency mice (lower IgE and eosinophilia level), leading to impaired parasite clearance.	Mice are used analogously to human beings. <i>Heligmosomoides polygyrus</i> infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.

Summary of Evidence for Hypothesis 2 Continued⁷

Host protein and/or energy deficiency (as measured by low HAZ/BMIZ/WAZ) impairs/suppresses the clearance of hookworm infection.								
Title (Author, Year)	Study Design	Findings	Notes	My conclusions	Animal Models, Clinical Trials, Intervention Studies			
					Experimental Design	Plausibility	Analogy	Specificity
The effect of protein deficiency on systemic release of rat mucosal mast cell protease II during <i>Nippostrongylus brasiliensis</i> infection and following systemic anaphylaxis. (Cummins et al., 1987)	animal study	Worm clearance has been prolonged in protein deficiency mice compared to normal diet mice (p <0.013).	analogous study using mice, primary infection	An animal study proved that protein-deficient mice have impaired clearance of <i>N. brasiliensis</i> .	An animal model using rats to study protein-malnutrition and <i>Nippostrongylus brasiliensis</i> (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. <i>Nippostrongylus brasiliensis</i> infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.
Evaluation of Biochemical, Hematological and Parasitological Parameters of Protein-Deficient Hamsters Infected with <i>Ancylostoma ceylanicum</i> . (Pacanaro et al. 2014)	animal study	Decreased production of IgG in low protein diet-fed hamsters led to increased hookworm burden.	Female hamsters were divided into negative control, negative infected, malnourished control, and malnourished infected groups. As a result, the experiments ended with 5, 6, 10 and 7 animals in 4 groups, respectively.	Compromised immune response was shown in low protein-fed hamsters (significantly lower IgG, p<0.01), hampering hookworm clearance.	An animal model using hamsters to study malnutrition and <i>A. ceylanicum</i> infection was established in this study.	Clinical manifestations of protein deficiency include weakness, decreased immune response, and increased susceptibility to invasion by pathogens. Compromised immune response was shown in low protein-fed hamsters (significantly lower IgG, p<0.01), hampering hookworm clearance.	Female hamsters are used analogously to human beings. <i>A. ceylanicum</i> infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.
The effect of iron and protein deficiency on the development of acquired resistance to reinfection with <i>Nippostrongylus brasiliensis</i> in rats. (Duncombe et al., 1979)	animal study	In this study, worm clearance has been prolonged in protein deficiency mice during secondary infection. But the important statement of the experiment is the protein deficiency stage is chronic rather than acute, and the infection dose of <i>Nippostrongylus brasiliensis</i> is small.	analogous study using mice and <i>N. brasiliensis</i> , secondary infection	An animal model proved that protein-deficient mice have impaired acquired resistance to reinfection of <i>Nippostrongylus brasiliensis</i> .	An animal model using rats to study protein-malnutrition and <i>Nippostrongylus brasiliensis</i> (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. <i>Nippostrongylus brasiliensis</i> infection was used analogously to hookworm infection.	Specificity was partial clear in this experimental animal study.
Delayed expulsion of the nematode <i>Nippostrongylus brasiliensis</i> from rats on a low protein diet: the role of a bone marrow derived component. (Duncombe et al., 1981)	animal study	In this study, worm clearance has been prolonged in protein deficiency mice.	analogous study using mice, primary infection	This animal model proved that protein-deficient mice have impaired clearance of <i>Nippostrongylus brasiliensis</i> .	An animal model using rats to study protein-malnutrition and <i>Nippostrongylus brasiliensis</i> (Nematoda) infection was established in this study.	Did not specifically mention in the study.	Mice are used analogously to human beings. <i>Nippostrongylus brasiliensis</i> infection was used analogously to hookworm infection.	Specificity was partial clear in this experimental animal study.
Effect of Nutritional Repletion on Human Hookworm Infection. (Tripathy et al. 1971)	clinical trails	No significant results obtained from the study showed that nutritional (protein) repletion influences the clearance of hookworm infection.	7 male and 4 female patients identified with malnutrition and heavy hookworm infections were enrolled in the study. They were treated with high protein diets after an initial period of the control diet. The degree of infection was assessed and monitored continuously till the end of the study period.	The difference between the overall hookworm counts before and after dietary treatment was not statistically different in this study.	Human subjects were recruited to study whether people with high protein food would have an impact on hookworm clearance.	Did not specifically mention in the study.	The study design is not applicable to assess analogy.	This criterion is rather fragile to assess the causal inference for the malnutrition-hookworm infection relationship. Specificity is somewhat clear in this clinical trial with limited information.

Summary of Evidence for Hypothesis 3

Host zinc deficiency impairs/suppresses the clearance of hookworm infection.								
Title (Author, Year)	Study Design	Findings	Notes	My conclusions	Animal Models, Clinical Trials, Intervention Studies			
					Experimental Design	Plausibility	Analogy	Specificity
Dietary protein and zinc restrictions independently modify a <i>Heligmosomoides polygyrus</i> (Nematoda) infection in mice. (Boulay et al., 1998)	animal study	Worm counts were significantly higher in low protein-fed mice and low-zinc fed mice. Parasite egg output was significantly higher in mice fed both low protein and low zinc diets. Significant lower eosinophilia was found in low-zinc fed mice.	an analogous study using mice and <i>Heligmosomoides polygyrus</i> (Nematoda)	Compromised immune response was shown in low zinc fed mice (significantly lower eosinophilia).	An animal model using mice to study dietary zinc restriction and <i>Heligmosomoides polygyrus</i> (Nematoda) infection was established in this study. This study combined 3 dietary protein levels and 2 dietary zinc levels during both primary infection and a challenging infection protocol.	Compromised immune response was shown in low zinc fed mice (significantly lower eosinophilia).	Infective 3rd-stage larvae (L3) of <i>H. polygyrus</i> were obtained by culturing the feces of chronically infected stock CD1 mice on moist filter paper for 7 days.	Under well and carefully control, specificity was clear in this experimental animal study.
Zinc deficiency impairs T cell function in mice with primary infection of <i>Heligmosomoides polygyrus</i> (Nematoda). (Shi et al., 1994)	animal study	The reduced production of IL-4 and IFN- γ , the reduced peripheral eosinophilia and reduced serum levels of IgE and IgG1 in Zn- mice were attributed to the zinc deficiency.	This study was designed to determine whether severe zinc deficiency would prolong the course of a primary <i>Heligmosomoides polygyrus</i> infection in mice, and whether this could be related to impaired T cell function.	In infected mice, Zn deficiency impairs production of IL-4 by Th2 cells in the GI tract via disruption of transcription, translation or transport of cytokines to the cell, thus decreasing Th2 production needed to clear infection. Also decreased IL-5 was found in zinc-deficiency mice. Dietary zinc deficiency influenced IgE, IgG1 and eosinophilia levels as shown by the significantly lower values in zinc deficiency mice.	An animal model using mice to study energy deficiency and <i>Heligmosomoides polygyrus</i> (Nematoda) infection was established in this study.	Suppressed T cell production and proliferation caused by zinc deficiency were shown in clinical and experimental studies. Also, one previous study showed that zinc deficiency has effect on host immune response, specifically T helper CD4 cell function. T helper cells are further classified into Th1 and Th2 cells, which are vital to hookworm clearance.	Mice are used analogously to human beings. <i>Heligmosomoides polygyrus</i> infection was used analogously to hookworm infection.	Under well and carefully control, specificity was clear in this experimental animal study.